Plant Physiol. (1988), 88, 1469–1473
0032-0889/88/88/1469/05/$01.00/0

H^+ Cotransports in Corn Roots as Related to the Surface pH Shift Induced by Active H^+ Excretion

Received for publication May 20, 1988 and in revised form August 8, 1988

JEAN-BAPTISTE THIBAUD, JEAN-CLAUDE DAVIDIAN, HERVÉ SENTENAC, ANDRÉ SOLER, AND CLAUDE GRIGNON*
Biochimie et Physiologie Végétales, Ecole Nationale Supérieure Agronomique, Institut National de la Recherche Agronomique (CNRS URA 198), 34060 Montpellier Cedex, France

ABSTRACT

The surface pH shift induced by active H^+ excretion in corn (*Zea mays* L.) roots was estimated using acetic acid influx as a pH probe (H Sentenac, C Grignon 1987 Plant Physiol 84: 1367–1372). At constant bulk pH, buffering the medium strongly reduced the magnitude of the surface pH shift. This was used to study the effect of surface pH shift on H^+ cotransports. In the absence of buffers, the surface pH shift increased with the bulk pH. Buffers decreased ^{32}Pi influx and this effect was stronger at pH 7.2 than at pH 5.8, and stronger in the absence than in the presence of an inhibitor of the proton pump (vanadate). Buffers exerted a similar depressive and pH-dependent effect on net NO_3^- uptake. They hyperpolarized the cell membrane, and stimulated $^{86}Rb^+$ influx, K^+:H^+ net exchange, and malate accumulation. These results are consistent with the hypothesis that H^+ accumulation at the cell surface is effective in driving H^+ reentry. We concluded that the surface pH shift due to proton pump activity is involved in the energetic coupling of H^+ cotransports.

Various nutrients are cotransported with H^+ across the plasma membrane of plant cells (8, 11). We have shown that H^+ excretion by corn root segments may maintain the apoplasmic pH well below the bulk medium pH (13). This phenomenon could increase the H^+ electrochemical potential gradient across the plasma membrane, thus contributing to energizing the various H^+-cotransport systems. The hypothesis of a surface pH shifted out of equilibrium by active proton pumping, which may drive NO_3^- and Pi transports, has already been put forward on the basis of some kinetic or thermodynamic features of the transports of these anions (14, 17, 18). Furthermore, the accumulation of H^+ at the cell surface could have a retroactive effect on the net K^+:H^+ exchange rate, since both net H^+ efflux and K^+ absorption are pH-sensitive (6, 7, 12, 15). We present here a study of the effects of the surface pH shift induced by H^+ excretion on Pi, NO_3^-, and K^+ absorption, and net H^+ transport by corn roots. The surface pH shift was evaluated using [2-^{14}C]acetic acid influx as a pH probe (13). It was modified, at constant bulk medium pH, either by inhibiting the proton pump or by driving the excess H^+ towards the bulk with buffers (9).

MATERIALS AND METHODS

Material Preparation and General Procedures. Corn (*Zea mays* L. cv INRA 508) seeds were surface sterilized with hypochlorite for 30 min. After germination between wet paper towels for 2 d, seedlings were grown on 0.2 mM $CaSO_4$ for 3 d at 25°C in the dark. Roots were excised below the first secondary roots, 5 cm from the seed. Excised roots were washed for 3 h in aerated 0.2 mM $CaSO_4$ before experiments. For ion transport measurements, the solutions were placed in a modified Büchner funnel fitted with a porous glass plate, through which CO_2-free air was bubbled. The pH was maintained with an automatic pH-stat system (Metrohm), which delivered 10 mN H_2SO_4 or 10 mN KOH as needed.

Influx Measurements. The influxes of ^{32}Pi, $^{86}Rb^+$ and [2-^{14}C] acetic acid were measured by adding the tracers to the absorption media 5 min after introducing the roots (0.6 g fr wt[1] in 100 mL). $^{14}CO_2$ released by the roots during [2-^{14}C]acetic acid uptake was trapped in 0.5 M ethanolamine. Tracer was incorporated for 10 min. Roots were then rinsed in ice-cold 0.2 mM $CaSO_4$ for 5 min (4 successive baths, 150 mL each), weighed, and finally treated with 20 mL of 0.1 M HCl for 24 h ($^{86}Rb^+$ and ^{32}Pi extraction) or with 6 mL of a tissue solubilizer (Soluene 350, Packard) for 72 h (^{14}C extraction). $^{86}Rb^+$ and ^{32}Pi were measured with a Packard liquid scintillator (Cerenkov effect). For ^{14}C counting, aliquots of Soluene root extracts (about 0.6 mL) were weighed and mixed with 15 mL of scintillation cocktail (5 g PPO and 0.1 g POPOP/L toluene); incubation media and ethanolamine solutions were sampled and prepared for liquid scintillation counting with Instagel (Packard). Influx of acetic acid was estimated by summing the ^{14}C contents of the Soluene extract and ethanolamine trap (13).

NO_3^- Net Uptake. Roots absorbed NO_3^- for 1 h, from solutions supplemented with 0.2 mM $Ca(NO_3)_2$ (3 g fr wt in 600 mL). They were then rinsed in 0.2 mM $CaSO_4$ for 5 min and weighed. NO_3^- was extracted with 20 mL of 0.1 M HCl for 24 h, and assayed by colorimetric determination of NO_2^-, after reduction in a cadmium column (4).

Net H^+ Flux, K^+ Absorption and Malate Synthesis. The solutions contained 2 g of fr wt roots per 100 mL. The pH was maintained at 6.0 ± 0.03. The net H^+ flux was calculated from the volumes of H_2SO_4 or KOH delivered for 4 h by the pH-stat (3). At the beginning and end of the experiments, root samples were rinsed for 5 min in 0.2 mM $CaSO_4$, weighed, frozen in liquid N_2, and ground. Organic acids and ions were extracted by adding 15 mL of ethanol and, 12 h later, 5 mL of water. K^+ and malate were assayed, respectively, by flame photometry and an enzymic method (3). Net K^+ uptake and malate accumulation were determined from differences between initial and final K^+ and malate contents.

Membrane Potential. This was measured classically, with a glass microelectrode inserted in a cortical cell as described elsewhere (15).

[1] Abbreviations: fr wt, fresh weight; J_{aa}, acetic acid influx; J_{Pi}, orthophosphate influx.

Vanadate. Sodium metavanadate (Merck) was dissolved for 12 h in water at 50 to 60°C and subsequently kept at room temperature as a 50 mM stock solution. Such a solution is as efficient in inhibiting plasma membrane Mg-ATPase (not shown) as the more often used orthovanadate solutions. Vanadate solutions prepared from ortho- or metavanadate give the same vanadate species in solution after equilibration (2).

RESULTS

Acetic Acid Influx. Washed roots were transferred to solutions containing 0.2 mM $CaSO_4$, 50 μM K_2SO_4, and 50 μM acetic acid labeled with [2-^{14}C]acetate. The pH was maintained by automatic titration at various values between 4 and 8. In parallel runs, the media were supplemented with 0.2 mM vanadate and/or with 5 mM buffers. The dependence of acetic acid influx (J_{aa}) on pH is shown in Figure 1A. J_{aa} diminished with increasing pH. Adding vanadate resulted in a decrease in J_{aa}, from 5% at pH 4.2 to more than 75% at pH > 6.0. Buffering the medium with Mes-Tris or Hepes-Tris in the pH 5.8 to 7.2 range decreased J_{aa} by approximately the same amount as did vanadate.

Net H^+ transports measured under the same conditions as J_{aa} are shown in Figure 2 in the pH 3.8 to 7.4 range, in the absence of buffers. In the absence of vanadate, a net efflux was observed, which increased with pH. In the presence of vanadate, the net efflux was reduced (pH > 6), or suppressed (pH about 6.0), or replaced by a net influx (pH < 6). The variation of net H^+ transport upon addition of vanadate was only slightly dependent on pH.

Pi Influx. ^{32}Pi influx was measured at two K^+ concentrations (0.1 and 10 mM), at pH 5.8 and 7.2. The effects of vanadate and/or of medium buffering were studied under these conditions (Table I). Increasing the pH inhibited J_{Pi}. This inhibition was higher in the presence of vanadate and/or buffers than in their absence. Buffering the medium decreased J_{Pi} at pH 7.2, but had a small (vanadate absent) or negligible effect (vanadate present) at pH 5.8.

The dependence of J_{Pi} on pH was further studied within the pH 4.0 to 8.0 range. Since corn roots preferentially transport $H_2PO_4^-$ (12), the Pi concentrations in the media were calculated to obtain the same bulk $H_2PO_4^-$ concentration (50 μM) at each pH value (Table II). This concentration was approximately 10-fold the apparent K_m of the Pi transport system (12). Pi influx kinetics in the presence and in the absence of vanadate are shown in Figure 3. Again, it was observed that the decrease in J_{Pi} when the pH rose above 6 was strongly dependent on the presence of vanadate and buffers.

NO_3^- Net Uptake. Induction of the high-rate NO_3^- absorption system (14) was obtained by washing the roots in 0.2 mM $Ca(NO_3)_2$ for 3 h. In other experiments (no induction), the washing was performed in the presence of 0.2 mM $CaSO_4$. The medium was then replaced by solutions containing 0.2 mM $Ca(NO_3)_2$, 50 μM or 5 mM K_2SO_4, and buffers if indicated (Table III). The pH was maintained at 5.8 or 7.2. In preliminary experiments, net NO_3^- uptake was estimated from NO_3^- depletion in the medium, and compared to NO_3^- accumulation. After 1 h in the presence of NO_3^- (no previous induction), the amounts of NO_3^- taken up and accumulated were the same. After induction, NO_3^- accumulation accounted for 75% of net NO_3^- uptake (the difference between net uptake and accumulation was probably due to NO_3^- reduction and transport into the xylem). As shown in Table III, buffering the medium systematically lowered NO_3^- accumulation. This effect was more pronounced at pH 7.2 than at pH 5.8, and greater before induction than after it. The net H^+ efflux was 4.5 to 5 μmol·h^{-1}·g^{-1} fr wt before induction, and 3 to 4 μmol·h^{-1}·g^{-1} fr wt after induction.

$^{86}Rb^+$ Influx, Net H^+ Transport, Net Malate Synthesis, and Membrane Potential Difference. Roots washed for 3 h in 0.2 mM $CaSO_4$ absorbed K^+ from solutions maintained at pH 6.0. These solutions were either buffered (5 mM Mes-Tris) or not. The K^+ concentration was 0.1 or 10 mM. Since corn roots acted as quasistoichiometric K^+:H^+ exchangers in these conditions (15), automatic titration of the medium with KOH ensured that both K^+ and H^+ concentrations remained constant. Mean values of net H^+ transport, K^+ accumulation rate, and net malate synthesis were estimated in 4-h experiments. K^+ influx was estimated from the amount of $^{86}Rb^+$ absorbed during a 10 min period starting 5 min after the beginning of the experiments. K^+ and H^+ transports, as well as malate production, were increased by similar amounts (about 1 μeq·h^{-1}·g^{-1}) when Mes-Tris was added to the medium (Table IV). The buffer produced a more negative membrane potential in the absence of vanadate (Table V). It had no significant electrical effect in the presence of this inhibitor.

In the above experiments, assays of root malate were used to monitor net carboxylate synthesis. In other experiments (results

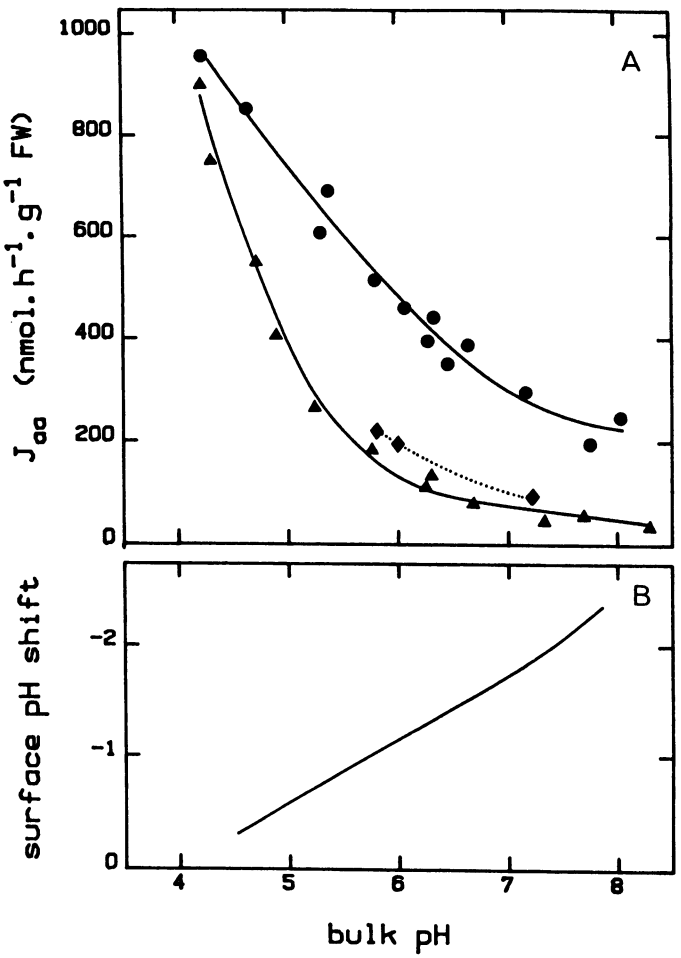

FIG. 1. Determination of the surface pH shift due to H^+ excretion. A, Effects of vanadate and pH buffers on acetic acid influx into corn roots. All media contained 0.2 mM $CaSO_4$, 50 μM K_2SO_4, and 50 μM acetic acid. Five KBq·ml^{-1} [2-^{14}C]acetic acid was added to the medium 5 min after the excised roots had been introduced into the medium. Incorporation was carried out for 10 min. When present, vanadate ($NaVO_3$) was at 0.2 mM. Buffered media contained 5 mM Mes-Tris (pH < 6.8), or 5 mM Hepes-Tris (pH > 6.8). The pH was kept constant with an automatic pH-stat system. The curves are polynomial fittings. (▲), Vanadate plus buffer; (♦), buffer alone; (●), neither vanadate nor buffer. B, Local pH shift due to H^+ excretion expressed as a function of the bulk external pH. The local pH shift was estimated from the shift of the J_{aa} curve along the x-axis due to addition of vanadate + buffer (A, polynomial fittings).

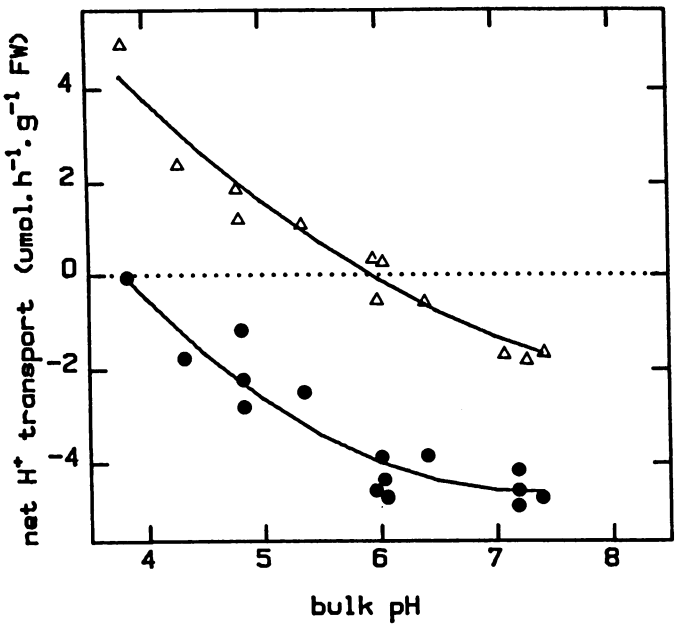

FIG. 2. Effect of pH on the net H^+ transports in the presence or absence of vanadate. Solutions contained 0.2 mM $CaSO_4$ and 50 μM K_2SO_4. When present, vanadate ($NaVO_3$) was at 0.2 mM. The mean net H^+ fluxes were calculated for 30 min intervals, from the recorded volumes of KOH or H_2SO_4 added by the automatic pH-stat to maintain pH at the chosen value (±0.03 pH unit). Negative values for net H^+ transport represent net efflux, positive values represent net influx. (●), No vanadate; (△), + vanadate. The curves are polynomial fittings.

Table I. *Effects of pH Buffers on Pi Influx in the Presence or Absence of Vanadate*

Incubation solutions contained 0.2 mM $CaSO_4$, 50 μM KH_2PO_4, 0.1 or 10 mM K^+ (K_2SO_4), pH 5.8 or 7.2. When added, vanadate ($NaVO_3$) was at 0.2 mM. Buffered solutions contained 5 mM Mes-Tris (pH 5.8) or 5 mM Hepes-Tris (pH 7.2). The pH was maintained by an automatic pH-stat system. ^{32}Pi (5 KBq·ml^{-1}) was added to the medium 5 min after the roots had been introduced, and incorporation was carried out for 10 min. Values are means of 5 or 6 experiments with 95% confidence limits.

Vanadate	Buffer	^{32}Pi Influx			
		0.1 mM K^+		10 mM K^+	
		pH 5.8	pH 7.2	pH 5.8	pH 7.2
mM		*nmol·h^{-1}·g^{-1} fr wt*			
0	0	351 ± 26	297 ± 16	360 ± 38	259 ± 30
0	5	321 ± 30	67 ± 9	303 ± 32	39 ± 2
0.2	0	254 ± 17	122 ± 9	250 ± 27	69 ± 8
0.2	5	257 ± 30	36 ± 4	268 ± 20	4 ± 3

not shown), net malate synthesis was found to be representative of net carboxylate synthesis. Four organic acids were identified by HPLC in ethanol extracts of washed roots, namely *trans*-aconitic, malic, quinic, and succinic acids. Malic acid accounted for 53% of the total amount. A 4-h treatment in the presence of 5 mM K_2SO_4 at pH 6.0, led to a 2- to 2.5-fold increase in the organic acid pool. The increase was 3-fold when 5 mM Mes-Tris was present. In all cases, the malic acid increase accounted for 79 to 82% of the organic acid pool increase.

DISCUSSION

On the assumption that vanadate affects J_{aa} only by changing the surface pH via inhibition of the proton pump (13), it is possible to estimate the surface pH shift due to H^+ extrusion. According to this hypothesis, the depressive effect of vanadate can be counteracted by acidifying the medium. Thus, each value of J_{aa} can be observed at two different pH values in the medium (with or without vanadate), and the surface pH shift due to active H^+ extrusion can be estimated from the difference between these two pH values. This procedure was applied to the data of Figure 1A. As shown in Figure 1B, the surface pH shift increased from 0.2 to more than 2 pH units when the bulk pH was varied from 4.2 to 8.

Two points must be emphasized before discussing the physiological aspects. The first one is the meaning of 'surface pH.' The surface pH is operationally defined by the type of probe used (here, membrane transport of acetic acid). This probe monitors the variations of the H^+ activity at a plane located at an unknown, but probably short, distance from the membrane. From this plane, the probe 'jumps' into the membrane without changing its ionization degree. This plane is probably inside the electrostatical diffuse layer, and thus the surface pH depends on electrostatical interactions with the fixed surface charges of the membrane, which may themselves be modified by various ionic treatments such as those we used. However, since we determined variations of J_{aa} upon vanadate addition, it is likely that the computed shifts in surface pH were due to variations of the H^+ excretion.

The second point is that pH cannot be modified by H^+ movement alone, because H^+ concentration is determined by the difference between concentrations of cations and anions. Thus, the pH gradient between the cell surface and the bulk must be associated to other ionic gradients. In our experiments, the acidification of the root surface was primarily due to K^+:H^+ exchange (15). Thus, the surface pH shift probably corresponded to accumulation of H^+ and depletion of K^+ at the cell surface, in response to membrane transport of these ions, and to restriction to their diffusion in the unstirred layers in the apoplast. Of course, it must be looked at as a mean value averaging all the radial and longitudinal heterogeneities of the root exchange surface (13).

Buffers with pKs close to the bulk pH suppressed the acidification of the cell surface. However, they stimulated net H^+ transport (Table IV). The diffusion of the protonated and nonprotonated species in opposite directions probably dissipated the H^+ concentration gradient across the unstirred layers at the root surface (9, 13). This reduction in the surface pH shift without inhibition of the proton pump made it possible to study the involvement of the root surface pH shift in coupling between active H^+ extrusion and other transport systems.

The data in Table I and Figure 2 indicate that buffers decreased J_{Pi} only when a net H^+ extrusion occurred, namely when vana-

Table II. *pH and Pi Concentration of the Solutions Used in the Experiments of Figure 3*

Pi was added as KH_2PO_4 at the indicated concentrations, to obtain 50 μM $H_2PO_4^-$ at all pH values. The concentrations were calculated as described in Ref. 12.

	Whole Pi Concentrations at the Indicated pH Values:							
	4.0	4.5	5.1	5.6	6.1	6.8	7.5	8.0
	μM							
Pi	50.8	50.6	50.6	51.4	54.2	70.6	153	375

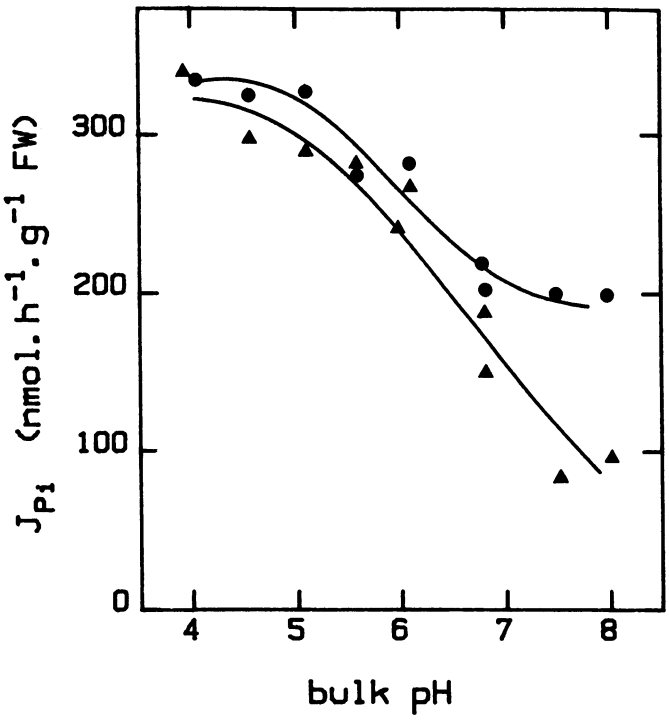

FIG. 3. Effects of pH on Pi influx in the presence or absence of vanadate and buffers. The solutions contained 50 μM K_2SO_4 and 0.2 mM $CaSO_4$. Pi was added as KH_2PO_4, to obtain 50 μM $H_2PO_4^-$ at all pH values (Table II). Five KBq·ml^{-1} ^{32}Pi was added to the medium 5 min after excised roots had been introduced. Incorporation was carried out for 10 min. When present, vanadate ($NaVO_3$) was at 0.2 mM, and the solutions were buffered (5 mM Mes-Tris for pH < 6.8, and 5 mM Hepes-Tris for pH > 6.8). Vanadate-free solutions were not buffered. The pH was kept constant with an automatic pH-stat system. (●), No vanadate nor buffer; (▲), vanadate and buffer present.

date was absent or ineffective in suppressing the H^+ efflux (pH 7.2). Thus, the depressive effect of buffers depended on the operation of the H^+ pump, and may not have resulted from a direct effect of buffers on the Pi transport system. Buffers hyperpolarized the membrane when added to vanadate-free media (Table V). However, this was probably not responsible for their effect on J_{Pi}, because this transport was only slightly sensitive to membrane potential: changing 0.1 mM K^+ to 10 mM K^+ strongly depolarized the membrane (Table V), but had a weak effect on J_{Pi} (Table I). J_{Pi} is known to decrease when pH is increased (6, 12). The simplest hypothesis is that buffers decreased J_{Pi} because they dissipated the pH gradient between the membrane surface and the bulk medium (Fig. 1A). As a corollary to this hypothesis, it can be proposed that the surface pH shift due to active H^+ extrusion is effective in stimulating J_{Pi}.

J_{Pi} is an uphill $H_2PO_4^-$ transport (1, 12) which is thought to be energized by H^+ cotransport (6). However, as discussed by Ullrich-Eberius *et al.* (17), the H^+ electrochemical gradient between bulk medium and cytoplasm is too weak to account for the measured Pi uptake above pH 6, even in the case of a 2 H^+:1 Pi symport. Additional restriction to Pi uptake at high pH is probably due to a decrease in the concentration of the transported form ($H_2PO_4^-$ varies from roughly 50% of total Pi at pH 7, to 10% at pH 8). It has thus been proposed that the H^+ pump provides H^+ cotransport systems with a local pH gradient (17), according to a model proposed by Kotyk (5). Our results provide evidence that such a coupling mechanism is at work in Pi transport. The quasiplateau of Pi uptake observed between pH 6 and 8, at a constant $H_2PO_4^-$ concentration and only in the absence of vanadate (Fig. 3, and Ref. 12), confirms that the Pi transport system did not respond to variation of bulk H^+ concentration when the H^+ pump was active.

NO_3^- uptake was depressed by buffers (Table III), although to a lesser extent than J_{Pi}. This transport is known to be stimulated at low external pH, and thought to be energized by the H^+ electrochemical gradient (14, 16). Thus, the above conclusion on the involvement of surface pH in the energetic coupling of Pi transport is extended to NO_3^- transport by the results of Table III. The fact that buffers decreased NO_3^- uptake less efficiently after induction of the high-rate NO_3^- transport system is consistent with the observed reduction in net H^+ efflux under this condition: rapid H^+ consumption by H^+:NO_3^- cotransport probably minimized the surface pH shift, and thus attenuated the sensitivity of NO_3^- uptake to buffers.

Buffers stimulated H^+ extrusion, $^{86}Rb^+$ influx, K^+ net flux, and malate accumulation (Table IV) and hyperpolarized the membrane (Table V). Thus, it can be concluded that surface accumulation of H^+ acted primarily by lowering electrogenic H^+ extrusion, which resulted in slowing down the associated carboxylations, and K^+ uptake as well. However, the results in Figure 2 indicate that the observed H^+ transport flux resulted from (a) vanadate-sensitive, pH-insensitive H^+ excretion; and (b) an H^+ influx that was highly dependent on pH, *i.e.* it increased when pH decreased. Thus, it is likely that H^+ accumulation at the cell surface resulted in an increase in H^+ reentry into the cells. For instance, the surface pH shift induced by H^+ extrusion amounted to about 1.2 pH units when the external bulk pH was 6.0 (Fig. 1B). Therefore, the surface pH was the same as in the presence of vanadate with the bulk pH adjusted to 4.8. Under these

Table III. *Effects of pH Buffer on Net NO^{3-} Accumulation*

Solutions contained 0.2 mM $Ca(NO_3)_2$ and 0.1 or 10 mM K^+ (K_2SO_4), pH 5.8 or 7.2. Buffered solutions contained 5 mM Mes-Tris (pH 5.8) or 5 mM Hepes-Tris (pH 7.2). pH was kept constant by an automatic pH-stat system. Absorption lasted 1 h. Induction: the high-rate NO_3^- transport system was induced by 3 h of pretreatment in 0.2 mM $Ca(NO_3)_2$. No induction: the pretreatment was performed in 0.2 mM $CaSO_4$. Means of 3 independent runs. From Student's *t* test for paired variates, the decrease in NO_3^- accumulation due to buffer addition was significant at pH 5.8 (P = 0.05) and at pH 7.2 (P = 0.02). Values in brackets are buffer effects expressed as % of control (no Mes-Tris).

Induction	Buffer	Net NO_3^- Accumulation			
		0.1 mM K^+		10 mM K^+	
		pH 5.8	pH 7.2	pH 5.8	pH 7.2
	mM	$\mu mol \cdot h^{-1} \cdot g^{-1}$ *fr wt*			
No	0	1.23	0.93	1.89	1.30
No	5	0.92 (−25%)	0.52 (−34%)	1.61 (−15%)	0.75 (−42%)
Yes	0	2.51	1.62	2.59	2.79
Yes	5	2.09 (−17%)	1.26 (−22%)	2.54 (−2%)	1.77 (−37%)

Table IV. *Effects of Mes-Tris on $^{86}Rb^+$ Influx, net K^+ Uptake, Net H^+ Transport, and Net Malate Accumulation*

The solutions contained 0.2 mM $CaSO_4$, and differed with respect to their K^+ (K_2SO_4) concentrations (as indicated) and the presence or absence of 5 mM Mes-Tris. pH was maintained at 6.0 ± 0.03 by an automatic pH-stat system. For $^{86}Rb^+$ influx measurements, the volumic tracer activity was 1 $KBq \cdot ml^{-1}$. The tracer was incorporated for 10 min. Net K^+ uptake, net H^+ transport and net malate accumulation were measured after 4 h (in this case, solutions were supplemented with 50 $mg \cdot L^{-1}$ chloramphenicol). Means of 7 independent runs with 95% confidence limits, or individual values.

	Fluxes in the Following Mediums			
	0.1 mM K^+		10 mM K^+	
	0 Mes-Tris	Mes-Tris 5 mM	0 Mes-Tris	Mes-Tris 5 mM
	$\mu mol \cdot h^{-1} \cdot g^{-1}$ *fr wt*			
$^{86}Rb^+$ influx	4.3 ± 0.47	5.5 ± 0.52	9.4 ± 0.35	10.0 ± 0.33
K^+ accumulation				
Experiment 1	2.7	3.8	3.9	4.9
Experiment 2	3.2	4.4	5.2	6.7
H^+ excretion				
Experiment 1	4.2	5.3	4.6	5.8
Experiment 2	4.5	6.2	5.9	6.8
Malate synthesis				
Experiment 1	1.0	1.3	1.2	1.6
Experiment 2	1.0	1.7	1.5	2.0

Table V. *Effect of Mes-Tris on the Membrane Potential Difference in the Presence or Absence of Vanadate*

The solutions contained 0.2 mM $CaSO_4$ and 0.1 or 10 mM K^+ (K_2SO_4), pH 5.8. When present, Mes-Tris was at 5 mM, and vanadate ($NaVO_3$) was at 0.2 mM.

Vanadate	Buffer	Membrane Potential Difference under the Following Conditions	
		0.1 mM K^+	10 mM K^+
mM		*mV*	
0	0	−130	−97
		−130	−100
0	5	−143	−112
		−150	−114
0.2	0	−117	−50
		−122	−56
0.2	5	−119	−51
		−120	−59

conditions (pH 4.8, vanadate present), the net H^+ influx was 1.2 $\mu mol \cdot h^{-1} \cdot g^{-1}$ fr wt (Fig. 2). This value is close to the increase in net H^+ efflux observed after addition of Mes-Tris (mean value: 1.4 $\mu mol \cdot h^{-1} \cdot g^{-1}$, Table IV).

In summary, active H^+ excretion associated with restriction to H^+ diffusion in the apoplast, attenuated the response of surface pH to variations of bulk pH: when the bulk pH was increased from 6.0 to 8.0, the surface pH was estimated to vary from 4.8 to 5.5 (from Fig. 1B). Since membrane potential (15) and cytoplasmic pH (10) are only slightly pH-dependent in the pH 6 to 8 range, it can be concluded that the surface H^+ accumulation prevents the membrane H^+ electrochemical gradient from collapsing as bulk pH is increased. Our results indicate that accumulation of H^+ at the cell surface contributes to the energetic coupling of transports, by stimulating both H^+ recirculation across the cell membrane, and associated cotransports.

Acknowledgments—We thank Denise Blayac for technical assistance.

LITERATURE CITED

1. BIELESKI RL 1973 Phosphate pools, phosphate transports and phosphate availability. Annu Rev Plant Physiol 24: 225–252
2. CHASTEEN ND 1983 The biochemistry of vanadium. Structure bonding 53: 105–138
3. DAVIDIAN JC, A SOLER, C GRIGNON 1984 Development of H^+ extrusion by barley roots after their excision. Physiol Veg 22: 163–170
4. HENRIKSEN A, AB SEMER-OLSEN 1970 Automatic methods for determining nitrate and nitrite reduction in water and soil extracts. Analyst 95: 514–518
5. KOTYK A 1979 Critic of coupled versus non coupled transport of non electrolytes. *In* A Kotyk, ed, Fifth Winter School on Biophysical Membrane Transport. School Proceedings, Vol 2, Academia, pp 50–67
6. LIN W 1979 Potassium and phosphate uptake in corn roots. Further evidence for an electrogenic H^+/K^+ exchanger and an OH^-/Pi antiporter. Plant Physiol 63: 952–955
7. LIN W, JB HANSON 1976 Cell potentials, cell resistance, and proton fluxes in corn root tissue. Plant Physiol 58: 276–282
8. POOLE RJ 1978 Energy coupling for membrane transport. Annu Rev Plant Physiol 29: 437–460
9. PRICE GD, MR BADGER 1985 Inhibition by proton buffer of photosynthetic utilization of bicarbonate in *Chara corallina*. Aust J Plant Physiol 12: 257–267
10. REID RJ, LD FIELD, MG PITMAN 1985 Effects of external pH, fusicoccin and butyrate on the cytoplasmic pH in barley root tips measured by ^{31}P-nuclear magnetic resonance spectroscopy. Planta 166: 341–347
11. REINHOLD L, A KAPLAN 1984 Membrane transport of sugars and amino acids. Annu Rev Plant Physiol 35: 45–83
12. SENTENAC H, C GRIGNON 1985 Effect of pH on orthophosphate uptake by corn roots. Plant Physiol 77: 136–141
13. SENTENAC H, C GRIGNON 1987 Effect of H^+ excretion on the surface pH of corn root cells evaluated by using weak acid influx as a pH probe. Plant Physiol 84: 1367–1372
14. THIBAUD JB, C GRIGNON 1981 Mechanism of nitrate uptake in corn roots. Plant Sci Lett 22: 279–289
15. THIBAUD JB, A SOLER, C GRIGNON 1986 H^+ and K^+ electrogenic exchanges in corn roots. Plant Physiol 81: 847–853
16. ULLRICH WR, A NOVACKY 1981 Nitrate-dependent membrane potential changes and their induction in *Lemna gibba* Gl. Plant Sci Lett 22: 211–217
17. ULLRICH-EBERIUS CI, A NOVACKY, E FISCHER, U LÜTTGE 1981 Relationship between energy-dependent phosphate uptake and the electrical membrane potential in *Lemna gibba* Gl. Plant Physiol 67: 797–801
18. ULLRICH-EBERIUS CI, A NOVACKY, AJE VAN BEL 1984 Phosphate uptake in *Lemna gibba* Gl: energetics and kinetics. Planta 161: 46–52